REMARKS

Claims 1-5, 7 and 9-18 are now in this application. Claims 1-5, 7 and 8 are rejected. Claim 9 had apparently not been examined. Examination of claim 9 is respectfully requested. Claim 6 is previously canceled and claims 2 and 8 are presently canceled. Claims 1-5, 7 and 9 are amended herein to clarify the invention and to address matters of form unrelated to substantive patentability issues.

The drawings are objected to for lacking proper labeling of Figs. 1a and 1b. Substitute formal drawings are provided herewith wherein the figures are labeled Fig. 1a, 1b and 2. Withdrawal of the objection to the drawings is respectfully requested.

The Office Action requests cooperation in correcting errors in the specification. Applicant submits herewith a substitute specification and abstract wherein amendments are effected to place the text thereof into proper English in accordance with 37 CFR 1.125(c). Also accompanying this amendment is a reproduction of the original specification and abstract with markings indicating the amendments effected in the substitute specification in accordance with MPEP §608.01(q) and 37 CFR 1.125(b). No new matter is added. Entry of the substitute specification and abstract is respectfully requested.

Claims

1. Ultrathin-walled multiwell plate for heat block thermocycling of samples comprising an array of small-volume wells of identical height with the similarly shaped sample wells formed in the top surface of the heat block of the thermocycler.

2. Ultrathin-walled multiwell plate according to claim 1, wherein the height of the wells of the plate is not more than the height of the sample wells formed in the top surface of the heat block of the thermocycler

3. Ultrathin-walled multiwell plate according to claim 1, wherein the walls of the wells are conically shaped.

4. Ultrathin-walled multiwell plate according to claim 1, wherein the thickness of the walls of the wells decreases from top to bottom.

5. Ultrathin-walled multiwell plate according to claim 1, wherein the wells of said multiwell plate are thermoformed into negative mould.

6. Ultrathin-walled multiwell plate according to claim 1, wherein the walls of the wells have an average thickness of 20-40 microns.

7. Ultrathin-walled multiwell plate according to claim 1, wherein the walls of the wells are deformable.

8. Ultrathin-walled multiwell plate according to claim 1, wherein the said microwell plate comprises a rigid supporting frame.

9. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is in the range of 16-85 μl.

Claims 1-5, 7 and 8 are rejected as indefinite under 35 U.S.C. § 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter of the invention as a result of informalities stated in the Office Action. The claim 1 is amended to address the informalities noted in the Office Action and claim 2 is canceled. With regard to the heat block, this item is now recited in the preamble to make clear it is not part of the body of the claim. Therefore, reconsideration of the rejection of claims 1-5, 7 and 8 and their allowance are earnestly requested.

Claims 1-5, 7 and 8 are rejected under 35 U.S.C. § 102(b) as being anticipated by Tretyakov. Applicant herein respectfully traverses these rejections. "Anticipation requires the presence in a single prior art reference disclosure of each and every element of the claimed invention, ***arranged as in the claim.***" ***Lindemann Maschinenfabrik GmbH v. American Hoist & Derrick Co.***, 221 USPQ 481, 485 (Fed. Cir. 1984) (emphasis added). It is respectfully submitted that the cited reference is deficient with regard to the following.

Claim 1 presently recites a rigid frame for supporting the tray outside of the heat block. Applicants further provide herewith German and English translations of the Tretyakov reference. According to the Tretyakov publication, a flat plastic film (2) is placed directly on the heat block (1) to begin with. The plastic film (2) is sealed by a peripheral gasket (3). The plastic film (2) itself does not have a

frame (4); the frame (4) is only the carrier of the gasket (3). The plastic film (2) is thermoformed by vacuum. By these means, the wells in the plastic film (2) are produced directly in the Thermocycler. For this purpose, evacuation channels are disposed in the heat block and, in each case, lead to the deepest points in the wells in the heat block (1). This plastic film (2) is very thin and it is not a carrier plate (multi-well plate) and can exist only in conjunction with the heat block. Without the heat block, the plastic film (2) would be deformed or destroyed. The plastic film (2) has several disadvantages:

1. The wells in the plastic film (2) can be filled with sample only in the Thermocycler, because the wells in the plastic film (2) are formed only in the heat block. It is not possible to fill the wells with sample outside of the Thermocycler.

2. The samples in the wells of the plastic film (2) can be evaluated also only in the Thermocycler. An evaluation of the samples outside of the Thermocycler is not possible, because the thin plastic film (2) cannot be removed without being destroyed or deformed.

3. This plastic film (2) is not suitable for a "rapid PCR", because, due to the channels for evacuating, the contact surface for the transfer of heat between the wells of the plastic film (2) and the wells of the heat block is reduced especially in the lower region of the wells (heat block). However, for rapid PCR, very small samples are preferred, which are to be found especially in the lower region of the wells of the heat block, in which however, the contact area is interfered with.

4. This plastic film (2) is also not suitable for rapid PCR, because ultra-thin film is not suitable for its formation; these films would be pulled into the channels during the evacuation or could easily be destroyed at the transition into the channels. However, for carrying out the rapid PCR, wells of a plastic film (2) with ultra-thin walls and little thermal isolation would be required in order to make rapid temperature changes possible.

The present invention overcomes these disadvantages. The claimed plate has wells with thin walls and a frame to support the plate. This plate can be inserted into the wells of the heat block. As a result, the plate can be handled

easily. It can be filled outside of the Thermocycler with samples. These samples can be evaluated later on outside of the Thermocycler in the plate. The plate therefore is "consumable". It holds its shape very well even without the support of a heat block. It can be prepared even without the heat block of a Thermocycler.

In view of the above, it is respectfully submitted that claims 1-5, and 7-9 particularly describe and distinctly claim elements not disclosed in the cited reference. Therefore, reconsideration of the rejections of claims 1-5, and 7-9 and their allowance are respectfully requested.

Claims 13-19 are added and are submitted as patentable for the combination of the plate having wells with wall thicknesses of 20 to 40 microns, a frame for supporting the plate, and a heat block having wells with apertureless bottoms for accepting the wells of the plate. This combination of features is not taught by the cited art.

Applicant respectfully requests a two month extension of time for responding to the Office Action. Please charge the fee of $410 for the extension of time to Deposit Account No. 10-1250.

In light of the foregoing, the application is now believed to be in proper form for allowance of all claims and notice to that effect is earnestly solicited. Please charge any deficiency or credit any overpayment to Deposit Account No. 10-1250.

Respectfully submitted,
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enc: Substitute Specification; Marked reproduction of original specification; replacement sheets for Figs. 1a, 1b and 2, and German and English translations of the Tretyakov publication.

MARKED SPECIFICATION

F-6954 Ser. No. 09/830,511

~~Ultrathin-walled multiwell plate for heat block thermocycling~~

ULTRATHIN-WALLED MULTIWELL PLATE FOR HEAT BLOCK THERMOCYCLING

BACKGROUND

The invention relates to plastic plates for conventional heat block thermocycling of biological samples, particularly to multiwell plates. More specifically, it relates to ultrathin-walled multiwell plates with an improved heat transfer to small-volume samples. Such plates can be used for rapid temperature cycling of multiple, small-volume samples (i.e. 1-20 μl) by using heat block thermocyclers with an increased block temperature ramping rate (i.e. 4° C/second and greater) and standard heated-lid technology for sealing the samples.

Temperature cycling of biological samples is a central moment in DNA amplification by the polymerase chain reaction (PCR) (Saiki et al., Science, 239, 487-491, (1988)). Much effort is being expended in developing various alternative reactors and technologies for rapid temperature cycling of small-volume samples (Kopp et al., Science 280, 1046-1048, (1998); Belgrader et al., J.Forensic Science 43, 315-319, (1998); Wittwer et al., Analytical Biochem.,

186, 328-331 (1990) and U.S. Patent No 5,455,175; Woolley et al., Analytical Chem., 68, 4081-4086 ((1996)).

One commercially available type of microreactor and thermocycler for rapid temperature cycling of small samples is a glass capillary tube and a hot-air thermocycler from Roche Molecular Biochemicals (cat No. 1909 339 and cat No. 2011468, respectively). The glass capillary tube can hold reaction volumes ranging from 10 to 20 µl. The hot-air thermocycler can hold 32 capillaries and perform 30 - 40 PCR cycles in 20-30 minutes. However, ~~these~~ this rapid DNA amplification technology ~~is connected with~~ has various disadvantages, for example:

a) The handling of the individual capillaries is relatively cumbersome.

b) The relatively large glass surface adsorbs components of the standard PCR-mixtures. This might inactivate the reaction. Therefore, various carrier molecules, i.e. proteins or even DNA, must be added and the concentrations of the components reoptimized.

c) The cost of the capillary tube, as a disposable PCR container, is high when compared to the standard 0.2 ml PCR tube.

d) The experimental throughput using this system is limited.

It is surprising that only little research has been conducted to improve the basic performance in sample size and speed of the widely used, conventional heat block thermocycling of samples contained in plastic tubes or multiwell plates.

_____One known improvement of heat block temperature cycling of samples contained in plastic tubes has been described by Half et al. (Biotechniques, 10, 106-112, (1991) and U.S. Patent No 5,475,610). They describe a special PCR reaction-compatible one-piece plastic microcentrifuge tube, i.e. a thin-walled PCR tube. The tube has a cylindrically shaped upper wall section, a relatively thin (i.e. approximately 0.3 mm) comically-shaped lower wall section and a dome-shaped bottom. The samples as small as 20 µl are placed into the tubes, the tubes are closed by deformable, gas-tight caps and positioned into similarly shaped conical wells machined in the body of the heat block. The heated cover to compresses each cap and forces each tube down firmly into its own well. The heated platen (i.e. heated lid) serves several goals by supplying the appropriate pressure to the caps of the tubes: it maintains the conically shaped walls in close thermal contact with the body of the block; it prevents the opening of the caps by increased air pressure arising in the tubes at elevated temperatures. In addition, it maintains the parts of the tubes that project above the top surface of the block at 95° -100° C in order to prevent water condensation and sample loss

in the course of thermocycling. This ~~made~~ makes it possible to exclude the placing of mineral oil or glycerol into the wells of the block in order to improve the heat transfer to the tubes and the overlaying of the samples by mineral oil that prevented evaporation but also served as added thermal mass. In addition, the PCR tubes can be put in a two-piece holder (US patent 5,710,381) of an 8x12, 96-well microplate format, which can be used to support the high sample throughput needs with any number between 1 and 96 individual reaction tubes.

In DE 4022792 the inventors describe a plate with cylindrically shaped walls of the wells and spherically shaped bottoms thereof. The individual wells of the plate were formed by melting a polycarbonate sheet in the range of 0.27-0.5 mm by a stream of hot air. This technology leads to relatively thin walls in the range of 0.08-0.2mm. The biological samples were placed into the wells, covered with polycarbonate film (0.1 mm) and the individual wells were thermosealed by a special press. Upon sealing the plate was placed on the thermoblock and fixed by screws. Though theoretically the heat transfer to the samples is improved, however, the way of positioning the plate on the block and the cylindrical and spherical geometry of the well prevent a close thermal contact with the heating block. During thermocyling, due to the large thermal expansion, the plate fixed by screws becomes deformed and the close

thermal contact is not maintained anymore. Therefore, by using the above technology rapid cycling reactions cannot be performed.

Another ~~The other~~ known improvement of heat block thermocycling is described in PCT patent application WO 98/43740 ~~. It~~ and concerns a heat block thermocycler with an increased ramping rate, i.e. 4° C/second ~~)~~. The thermocycler can hold 96 PCR tubes (each of a volume of 0.2 ml) or 96-well PCR plates. Theoretically, the thermocycler can perform 30 PCR cycles in 20-30 minutes, provided that only a few seconds are spent to reach the temperature equilibrium between the heat block and the samples.

However, as described in U.S. Patent No 5,508,197, even if the temperature of the heat-transfer media, i.e. water, is changed almost instantaneously, it takes approximately 15 seconds to reach equilibrium between water and the 15-20 µl samples in the standard PCR plates. This means that for 30 PCR cycles approximately 20 minutes are spent to reach the equilibrium between heat-transfer media and the 15-20 µl samples in the plates.

In comparison, the above mentioned heat block cycler (WO 98/43740) operating at a ramping rate of 4° C/second, needs for the heat-block temperature transitions during 30 PCR cycles 10 minutes only. This shows that the major limiting factor for rapid temperature cycling of small samples in ~~platic~~ plastic

PCR tubes or PCR plates is the low efficiency of the heat transfer through the walls of conventional PCR tubes or plates, respectively.

SUMMARY

The present invention concerns plastic multiwell plates for performing heat block thermocycling of multiple samples. More specifically, it concerns ultrathin-walled multiwell plates with an improved heat transfer to small samples. Ultrathin-walled multiwell plates are suited for rapid, oil-free, heat block temperature cycling of small volume samples (i.e. approximately 1-20 µl), whereas the lower limit is given by the reliability of the conventional pipetting systems.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a is a plan view of an embodiment of ~~Figure 1 illustrates an example of~~ a multiwell plate according to the present invention.

Fig. 1b is a cross-sectional side elevation view of the multiwell plate of Fig. 1a.

Fig. 2 is a cross-sectional side elevation view of the multiwell plate of Fig. 1a positioned ~~Figure 2 illustrates the positioning of the plate in the~~ in a block of ~~the~~ a thermal cycler.

DETAILED DESCRIPTION

One aspect of the present invention concerns ~~the~~ a multiwell plate having a considerably decreased thickness (i.e. approximately 7.5-15 fold) of ~~the~~ well walls when compared to known thin-walled PCR tubes (U.S. Patent No 5,475,610). This can be ~~reached~~ achieved, for example, by means of thermoforming the multiwell plates out of thin thermoplastic films. Such thermoplastic films are, for example, polyolefin films, such as metallocene-catalyzed polyolefin films and/or copolymer films. Usually, the multiwell plate is vacuumformed out of cast, unoriented polypropylene film, polypropylene-polyethylene copolymer films or metallocenecatalyzed polypropylene films. The film is formed into a negative ("female") mould comprising a plurality of spaced-apart, conically shaped wells which are machined in the body of a mould in the shape of rectangular- or square-array. The thickness of the film for vacuumforming conically shaped wells is chosen according to the standard rule

used for thermoforming, i.e. thickness of the film = well draw ratio x thickness of the wall of the formed well.

For example, vacuumforming wells with a draw ratio of two and an average thickness of the walls of 30 microns ~~results in~~ requires a film thickness of 60 microns. The average optimum wall thickness was found to be 20-40 microns. The thickness of the well is reduced 7.5-15 fold when compared to the wall thickness of the formerly improved PCR tube ~~desribed~~ described in U.S. Patent No 5,475,610. Using the Fourier equation for heat transfer and the equation for temperature transfer through solid substances, it can be shown that heat transfer through one square millimeter of the surface of the well of the plate is increased 7.5-15 fold and the time of temperature transfer through the wall is decreased 56-225 fold when compared to the ~~said~~ PCR tube. This drastic decrease in time can be explained by the fact that the time needed for the transfer of temperature front is proportional to the square power of distance. It can be easily calculated that the time of the temperature transfer through the ultrathin walls of the multi-well plate is in the range of milliseconds, whereas for the ~~said~~ PCR tube (U.S. Patent No 5,475,610) it is in the range of seconds. This explains the well known fact that thin (20-40 microns) plastic films are poor theremo insulators.

The thickness of the walls of the formed wells is gradually reduced to the bottoms of the wells due to vacuumforming of the wells into a negative mould. This geometry of the walls of the wells provides several advantages:

The relatively thick upper parts of the walls of the wells cause additional rigidity of the whole multiwell plate.

During heating of the heat block of the thermocycler, a vertical temperature gradient is formed in the sample, due to the gradient of the well-wall thickness. This vertical temperature gradient causes intensive convective mixing of the sample in conically shaped wells and increases the heat transfer through the sample. In comparison, this convective mixing of the sample is much less efficient in conventional PCR plates/tubes with a uniform wall thickness.

Another aspect of the invention concerns the height of the wells of the multiwell plate. The height of the conically shaped wells is equal to the height of the similarly shaped sample wells machined in the body of the heat block. Thus, this geometry of the wells (2) enables the positioning of the plate (1) on the heat

block (4) as shown in Figure 2. As shown (Figure 2), in contrast to the conventional PCR plates, the walls of the wells (2) of the multi-well plate (1) do not project above the top surface of the block (4). The type of positioning provides several advantages: The pressure caused by the screw (12) to the lid (10) (heating element (11)) can be increased in order to obtain efficient sealing of the samples (9) sealed, for example, by a silicon mat (13). In this case the pressure is to actually directed to those parts of the multiwell plate (1) which are supported by the top surface of the heat block (4) (or by parts of the top surface surrounding individual wells depending on the geometry of the heat block) and not to the thin walls of the wells of the plate as it is the case for the PCR tubes or conventional PCR plates. This advantage makes it possibe to increase the sealing pressure of the heated lid (10) several fold when compared to the conventionally used pressure of 30-50 g per well without cracking the conically shaped walls of the wells (2).

The extremely thin walls of the wells, i.e. 20-40 microns, are highly flexible as the multiwell plates are thermoformed out of highly elastic films (or sheets depending on the draw ratio). The walls of the wells are highly resistant against stress cracking, due to their flexibility and elasticity. As the wells of the plate, positioned on the heat block, are tightly sealed at room temperature, the air pressure in the wells will increase at elevated temperatures. The increased air

pressure causes a deformation of the walls of wells and brings them in tight thermal contact with the surface of the walls of the individual sample wells machined in the body of the heat block. Standard PCR plates (having relatively thick and rigid walls of the wells) require that the conically shaped walls of the wells have to match perfectly with the shape of the wells machined in the body of the heat block to guarantee a close thermal contact (see for example U.S. Patent No 5,475,610). This requirement is not as critical for the ultrathin walled multiwell plates of the invention, due to flexibility and elasticity of the walls of the wells. Using this advantage, special shapes of both, the walls of the wells of the plate and the wells of the heat block can be differently designed. These differently designed wells can promote an even closer thermal contact after positioning the plate into the heat block.

Another aspect of the invention concerns the frame of the multiwell plates. As the plates can be formed of very thin films (depending on the draw ratio of the well; supra) the flexibility of, for example, standard-format plates, i.e. 96-well PCR (8,5 x 12,5 cm) plates, is such that handling is not easily possible anymore. Therefore, depending on the geometry of the plate, a supporting frame might be needed, for example for industry standard formats, i.e. 96-, 192-, 384-well PCR plates. This frame can support, for example in case of small plates, the edges of the plate, or individual wells of the plate, or

groups of wells. For handling with robots, for example, the frame can be injection molded in the form of the standard skirted microplates containing the array of holes in the top surface of the frame matching the array of wells of the ultrathin multiwell plate. The plate can be attached to the frame by for example heat bonding. However, for small format plates including the frame can be formed as a single piece by using specially designed moulds.

The polypropylene-based plastics are PCR-compatible and therefore widely used for injection molding of PCR tubes and/or multiwell plates. In addition, they are resistant to stress cracking and have a reduced water vapor sorption when compared to other plastics (e.g. polycarbonate). Such plates can be thermoformed in both, standard industry formats, i.e. 96-, 192and 384-well PCR plates for large scale applications, supported by robots and small foot-print formats to match small foot-print thermocyclers, i.e. "personal thermocyclers".

The following example serves to illustrate the invention but should not be construed as a limitation thereof.

Example:

Fig. 1 illustrates a 36-well ultrathin walled multiwell plate according to the invention. The plate was designed for rapid temperature cycling of samples

ranging from 0.5-4 μl using a small foot-print pettier-driven heat block thermocycler supplied with a "wine-press" type heated lid (Fig. 2). The volume of the wells is 16 μl and the distance between the wells is 4.5 mm, i.e. industry standard for high sample density 384-well PCR plates. The diameter of the openings of the wells is 3.8 mm and the height of the wells is 3 mm. The average thickness of the walls of the wells is 30 μm. The frame (3) was cut out of a polypropylene sheet of a thickness of 0.5 mm and heat bonded to the plate (1). The area of the plate (1) is 30 x 30 mm. As shown in Figure 1, the handling of the plate (1) containing the multiple wells (2) is facilitated, by a rigid 0.5-1 mm thick plastic frame (3) which is heat bonded to the plate. As shown in Figure 2, the frame (3) is not in direct thermal contact with the block (4) during thermocycling because the inner contour (5) of the frame (3) matches the outer contour (6) of the heat block (4) of the thermocycler (7 = thermoelectric heat pump and 8 = air-forced heat sink).

The ultrathin walled multiwell plate according to the invention (Fig. 1) was experimentally tested for the amplification of a 455-base pairs long fragment of human papilloma virus DNA. The sample volume was 3 μl. For various PCR reactions, the average ramping rate of the thermo cycler was varied from 4° C to 8° C per second. The samples (i.e. standard PCR-mixtures without any carrier molecules) were transferred into the wells of the plate by

means of conventional pipetting equipment. The plate was covered by standard sealing film (Microseal A; MJ-Research, USA), transferred into the heatblock of the thermocycler and tightly sealed by the heated lid as shown in Fig. 2. Upon sealing, a number of 30 PCR cycles was performed in 15-25 minutes depending on the ramping rate of the thermo cycler. The PCR product was analyzed by conventional agarose electrophoresis. The 455-base pairs long DNA fragment was amplified with a high specificity at the indicated ramping rates (supra).

Plates according to the invention with well volumes of 35 µl were successfully tested for temperature cycling of samples of a volume of 20 µl . Thereby, 30 PCR cycles were performed in 20-30 minutes at a ramping rate of 6° C per second. Surprisingly, although the average thickness of the walls was 20 microns and the volume of the wells was 35 µl , samples of a volume of as few as 0.5 µl can be easily amplified without reducing the PCR efficiency.

In conclusion, the ultrathin walled multiwell plates according to the invention, allow a simple and rapid loading of multiple samples by conventional pipettes, rapid sealing of all samples by using conventional sealing films and rapid DNA amplification (15-30 minutes for 30 cycles) with an improved specificity typical for rapid cycling (Wittwer et al., Analytical Biochem., 186,

328-331 (1990)) using appropriate heat block thermocyclers (i.e. ramping rate in the range of 4° C to 8° C per second).

~~Claims~~ What is claimed is:

~~Ultrathin-walled multiwell plate for heat block thermocycling~~

Abstract of the disclosure

~~Ultrathin-walled~~ An ultrathin-walled multiwell ~~reactors~~ reactor for heat block thermocycling of samples ~~comprising~~ includes an array of small-volume wells of identical height with similarly shaped sample wells formed in ~~the~~ a top surface of ~~the~~ a heat block ~~of the thermocycler are provided~~. The multiwell plates are preferentially ~~vacuumformed~~ vacuum formed out of a 30-50 micron thick thermoplastic film and can be used for rapid, oil-free temperature cycling of small (1-10 μl) volume samples.

Bioorganische Chemie, 1997, vol. 23, No. 2, pages 526 – 528

Letter to the Editor

A new method for the rapid amplification of DNA amplification with the help of PCR in miniaturized, ultra-thin micro-well plates, which are formed directly on the heating block of the Thermocycler, was developed. These micro-well plates are produced from thin (40 – 60 μm) plastic film by hermetic vacuum molding.

Due to the efficient heat transfer to the samples (10 – 15 μL), rapid heating and cooling of the Thermocycler (up to 7°/c), the time required for DNA amplification within 30 cycles is 15 – 30 minutes.

Key words: polymerase chain reaction, DNA amplification, micro-well plate

RAPID DNA AMPLIFICATION IN MINIATURIZED, ULTRA-THIN MULTI-WELL PLATES

The cyclic incubation of the reaction mixture (sample) with a subsequent temperature change, which proves to be optimum for DNA synthesis, liquefaction of synthesis products and separation of primers, is an essential step in DNA amplification with the help of the PCR method. When the special technology of rapid DNA amplification (3, 4) is used, it is possible to shorten the PCR time by a factor of 5 to 10. At the same time, however, the sensitivity and specification of the method is increased in comparison to conventional methods (5 – 7).

In this report, a new method of rapid DNA amplification is described, which is applied in minitiaturized, ultra-thin multi-well plates on the heater block of a Thermocycler. A thin (40 – 60 μm) plastic mat (film) is fastened to the heater block of the Thermocycler with a rectangular frame with a rubber seal. This plastic mat

(sealing mat) is heated to the melting point (145°C) and molded by the resulting **discharge**? of about 0.8? in the cavity between the mat and the surface of the heating block. Multi-well plates, molded in this manner, are a fifth to a tenth as thick as thick-walled reaction vessels (0.5 mL) for PCR reactions, intended for this purpose. Since polypropylene is distinguished by its low adhesion to metals, the plastic mat does not adhere to the heater block. Special fastening prevents deformation of the multi-well plate prevents deformation of the multi-well plate during the filling and removal of the samples.

The multi-well plate consists of 96 wells (sample volumes of 30 μL), is 40 x 50 mm in size and therefore a quarter the size of a standard plate with the same number of wells. The distance between the wells is 4.5 mm. Pipetting can be carried out with conventional multi-channel pipettes. During the PCR, the multi-well plate is pressed by the resulting **discharge** close to the surface of the heater block. This ensures an efficient heat transfer from the thermal block to the reaction mixture (sample) (10 – 15 μL). In order to avoid loss of liquid from the reaction mixture during the PCR, the multi-well plate is covered hermetically with a film (Microseal "A" film, MJ Research, Inc. USA). This film is pressed with a heater lid, heated to 75°C, against the heater block. Standard hybridization with mineral oil is also possible.

Figure 2 shows amplification results of DNA fragments in HIV-1, HPV-18 and *ApoB* within 30 cycles in 20 minutes. The PCR was carried out in primer systems, which have already been mentioned (8 – 10). A plasmid with cDNA of the HIV-1 (for the amplification of the DNA fragment of the HIV-1) and DNA of the HeLa cells (for HPV-18 and *ApoB*) were (used) as matrices.

Reaction mixture (15 μL): 67 μm of tris-HCl (pH 8.8), 16.6 μm of ammonium sulfate, 7 μm of magnesium chloride, 0.01% of Tween 20, 0.25 μm per dNTP, 0.3 ? per primer.

To the mixture, 2.5 **units** of Taq DNA polymerase (Fermentas, Lithuania), 1? plasmid DNA or 50? DNA of the HeLa cells were added.

Even during the short duration of synthesis, liquefaction and separation, the high number of PCR products is ensured (only 1/30 of the amount of the reaction mixture was used for the electrophoresis). This means that products, which inhibit the PCR, are not formed by the molding of the micro-well plate at a high temperature.

Three different primer systems did not show any formation of unusual products during the rapid DNA amplification (no details given).

the temperature change of the sample, which is slow under the conditions, the DNA amplification at different temperatures the separation and concentrations of the ions of Mg ensured *(sic!)*

All of this simplifies the selections of the conditions for simultaneous PCR throughput with different primers.

When this method is used (in comparison to those employed previously), it is possible to automate and simplify the process of pipetting the 96 multi-well plate with conventional multi-channel pipettes. The technology of rapid DNA amplification, which has been developed, can find very broad applications in PCR analyses (sequencing and mapping, mass PCR screening of pathogens, etc.).

In theory, it is possible to carry out more than 1,500 reactions in one working day.

Figure 1

Diagrammatic shaping of the ultra-thin micro-well plate for PCR

1. Heater block
2. Plastic film
3. Rubber seal
4. Rectangular frame
5. Vacuum connection
6. Reaction mixture
7. Hermetic film
8. Heater lead

Figure 2

Electrophoresis of the amplified DNA fragments of the HIV-1 (1), HPC-18 (3) and *ApoB(4).*

1. Marker (ββββ

The standard length was given

Amplification conditions

93°C – 30c; following 30 cycles: synthesis 70°C, 15c; liquefaction - 93°C, 5c; separation: 52°C, 3c. The electrophoresis was carried out in 1.7% agarose gel, 0.089M tris buffer (1 μm of EDTA and 0.5 μg of ethidium bromide).

Bioorganische Chemie, 1997, Band 23, Nr. 2, Seiten 526-528

Brief an den Redakteur

Eine neue Methode der schnellen DNA-Amplifikation mit Hilfe von PCR in miniaturisierten, ultradünnen Mikrowell-Platten, die unmittelbar auf dem Hitzeblock des Thermocyclers geform werden, wurde entwickelt. Diese Mikrowell-Platten werden aus dünner (40-60 µm) Kunststofffolie durch hermetische Vakuumformung hergestellt.
Dank effizienter Wärmeübertragung zu den Proben (10-15 µl), schneller Aufheizung und Abkühlung des Thermocyclers (bis zu 7° C/c), beträgt die Zeit, die für DNA-Amlifikation innerhalb von 30 Zyklen notwendig ist, 15-30 Minuten.

Schlüsselwörter: Polymerasekettenreaktion, schnelle DNA-Amplifikation, Mikrowell-Platte

Schnelle DNA-Amplifikation in miniaturisierten, ultradünnen Multiwell-Platten

Wesentlicher Moment (Schritt) der DNA-Amplifikation **mit Hilfe von (mittels)** PCR-Methode besteht in der zyklischen Inkubation der Reaktionsmischung (Probe) mit nachfolgendem Temperaturwechsel, der sich für DNA-Synthese, Schmelzung von Syntheseprodukten und Abtrennung von Primer als optimal erweist.
Bei der Anwendung spezieller Technologie der schnellen DNA-Amplifikation [3,4] ist es möglich, die PCR-Zeit um 5-10-mal zu verkürzen, dabei aber ihre Empfindlichkeit und Spezifikation, im Vergleich zu üblichen Methoden, zu erhöhen [5-7].

In diesem Bericht wird eine neue Methode schneller DNA-Amplifikation beschrieben, die in miniaturisierten, ultradünnen Multiwell-Platten auf dem Hitzeblock eines Thermocyclers [4] angebracht werden. Eine dünne (40-60µm) Kunststoffmatte (Folie) wird mit einem rechtwinkligen Rahmen mit Gummidichtung auf dem Hitzeblock-Thermocycler befestigt. Diese Kunststoffmatte (Dichtungsmatte) wird bis zum Schmelzpunkt (145° C) erwärmt, und durch entstehende **Entladung**? von ca. 0.8? im Hohlraum zwischen der Matte und der Oberfläche des Hitzeblocks, geformt. Auf diese Weise geformte Multiwell-Platten sind 5-10-mal dünner als dafür vorgesehene dickwandige Reaktionsgefäße (0,5ml) für PCR-Reaktionen. Da sich Polypropylen durch niedrige Adhäsion gegenüber Metallen auszeichnet, haftet die Kunststoffmatte nicht am Hitzeblock. Spezielle Befestigung verhindert Deformierung der Multiwell-Platte während der Füllung und Entnahme der Proben.
Die Multiwell-Platte besteht aus 96 Wells (Probenvolumina 30µl), ist 40*60 mm groß und damit 4-mal kleiner als eine Standardplatte mit der selben Anzahl der Wells. Die Entfernung zwischen den Wells beträgt 4,5 mm. Pipettierung kann mit üblichen Mehrkanalpipetten gemacht werden. Die Multiwell-Platte wird während der PCR durch entstehende **Entladung** dicht an die Oberfläche des Hitzeblocks gepresst, was eine effiziente Wärmeübertragung vom Thermoblock zu der Reaktionsmischung (Probe) (10-15µl) sichert. Um Flüssigkeitsverlust der Reaktionsmischung während der PCR zu vermeiden, wird die Multiwell-Platte mit einer Folie hermetisch abgedeckt (Microseal "A" Film, MJ Research, Inc., USA). Diese Folie wird mit einem Heizdeckel (auf 75° C erwärmt) an den Hitzeblock gepreßt. Möglich ist auch die Standardhybridisierung mit Mineralöl.

Die Abbildung 2. zeigt Amplifikationsergebnisse von DNA-Fragmenten in HIV-1, HPV-18 und *ApoB* innerhalb von 30 Zyklen in 20 min. PCR wurden in Primersystems, die bereits erwähnt worden sind [8-10], durchgeführt. Als Matrize wurde ein Plasmid mit cDNA des HIV-1 (für die Amplifikation des DNA-Fragmentes HIV-1) und DNA der HeLa-Zellen (für HPV-18 und *ApoB*).
Reaktionsmischung (15 µl): 67 µm Tris-HCL (pH 8.8), 16,6µm $(NH_4)_2SO_4$, 7µm $MgCl_2$, 0,01% **Tween 20**, 0.25 µm pro dNTP, 0,3 ? je Primer.
Zu der Mischung wurden 2,5 **Einheiten** Taq-DNA-Polymerase (Fermentas, Litauen), 1? Plasmid-DNA oder 50? DNA der HeLa-Zellen zugegeben.

Sogar während der kurzen Dauer der Synthese, Schmelzung und Abtrennung wird die hohe Anzahl der PCR-Produkten gewährleistet (für die Elektrophorese wurde nur 1/30 der Reaktionsmischungmenge verwendet). Das heißt, dass bei der **Formierung** der Mikrowell-Platte unter Hochtemperatur, keine Produkte, die PCR **inhibieren**, gebildet werden.

Drei verschiedene Primersysteme zeigten keine Entstehung von unüblichen Produkten während der schnellen DNA-Amplifikation (ohne Angaben).

die unter den Bedingungen langsamer Temperaturänderung der Probe die DNA-Amplifikation bei unterschiedlichen Temperaturen der Abtrennung und Konzentration der Ionen Mg gewährleisteten

All das vereinfacht die Auswahl der Bedingungen für gleichzeitige PCR-Durchsätze mit unterschiedlichen Primer.

Bei der Anwendung dieser Methode (im Vergleich zu früher angewendeten) ist es möglich, Pipettierugsprozess der 96-Multiwell-Platte mit üblichen Mehrkanalpipetten zu automatisieren und zu vereinfachen. Die entwickelte Technologie der schnellen DNA-Amplifikation kann sehr breite Anwendung in PCR-Analysen finden (Sequenzierung und Mapping, Massen-PCR-Screening von Patogenen usw.).

Theoretisch ist es möglich, mehr als 1500 Reaktionen innerhalb eines Arbeitstages durchzuführen.

Abb. 1

Schematische Formung der ultradünnen Mikrowell-Platte für PCR:

1. Hitzeblock
2. Kunststoffolie
3. Gummidichtung
4. rechtwinkliger Rahmen
5. Vakuumanschluß
6. Reaktionsmischung
7. hermetische Folie
8. Heizdeckel

Abb. 2

Elektrophorese der amplifizierten DNA-Fragmenten des HIV-1(1), HPV-18 (3) und *ApoB (4)*.

1. Marker (ßßßß

Standardlänge wurde angegeben.

Amplifikationsbedingungen:

93° C-30c; folgende 30 Zyklen: Synthese-70° C, 15c; Schmelzung:-93°C, 5c; Abtrennung-52°C, 3c. Elektrophorese wurde in 1.7% Agarosegel, 0.089 M Tris-Puffer (1μm EDTA und 0,5 μg Ethidiumbromid) durchgeführt.